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Diagnostic Efficacy of ERG and CK5 Coexpression in Prostatic Intraepithelial Neoplasia and Carcinoma

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Abstract

Background: *Prostate lesions either benign, Premalignant or malignant, continues to be distressing and annoying problem for old people from centuries. Carcinoma of prostate is most common internal malignancy among men & is responsible for 10% of cancer death in the population. The combination of ERG and CK5 provides a unique stain that identifies the TMPRSS2-ERG chromosomal translocation in prostate cancer. The current study aims to detect the diagnostic efficacy of ERG & CK5 co-expression in BPH, PIN & Prostatic carcinoma.*

Aims & Objectives: *Study of coexpression of ERG & CK5 immunohistochemical stain in BPH, PIN and Prostatic carcinoma and to correlate with serum PSA level, tumor stage and grade.*

Materials & Methods: *We received TURP and Tru-cut biopsy specimen for histopathological examination. Specimens were processed, sections cut, stained with Hematoxylin & Eosin as well as apply cocktail of ERG & CK5 immunohistochemical stain as per standard protocol.*

Results & Discussion: *On the basis of histology, 11 cases were Benign Prostatic Hyperplasia (37%), 9 cases were Benign Prostatic Hyperplasia with Prostatic Intraepithelial Neoplasia (30%) and 10 cases (33%) were prostatic carcinoma.*

Sensitivity and specificity of ERG&CK5 immunostain in detecting ERG positive prostatic adenocarcinoma being 70% and 100% respectively in our study. Thus staining for ERG&CK5 has great utility in resolving diagnostic problems of PIN and adenocarcinoma prostate cases that arises in prostatic needle biopsies containing small foci of suspicious cells.

Conclusion: *However immunohistochemical stain is an important adjunct to the diagnosis and predictive analysis of prostate biopsy specimens.*

Keywords: *Immunohistochemical stain, ERG, CK5, TURP & Tru-cut biopsy of Prostate.*

Introduction

Carcinoma of the prostate is the most common internal malignancy among men.[1] Prostate cancer is the leading cause of cancer in men and is second only to lung cancer as a leading cause of cancer related death in men. Prostatic intraepithelial neoplasia (PIN) is the preferred term for a process involving prostatic ducts and acini which has also been described as intraductal or ductal-acinar dysplasia.[2] ERG oncoprotein is the most common of the transcription factors that is produced as a consequence of the many gene fusion events that affects the regulation of androgen receptor prostate associated genes[3]. ERG oncoprotein is a promising diagnostic marker for identifying prostatic adenocarcinoma and distinguishing it from non-neoplastic prostate and other carcinoma.

CK5 stains normal basal cell layers in normal glands, benign glands (Benign Prostatic Hyperplasia (BPH) and prostatic intraepithelial neoplasia (PIN).The combination of ERG and CK5 provides a unique stain that identifies the TMPRSS2-ERG chromosomal translocation in prostate cancer,and also helping to visualize ERG positive PINs. The current study aims to detect the diagnostic efficacy of ERG & CK5 co-expression in PIN & prostatic adenocarcinoma.

Aims & Objectives

To study the coexpression of ERG &CK5 gene immunostain in BPH, PIN and prostatic carcinoma and to correlate with serum PSA level, tumor stage and grade.

Materials & Method

The present study was conducted in the department of pathology in collaboration with the department of urology. In this study we included clinically suspected or diagnosed cases of benign hyperplasia of prostate, carcinoma of prostate and prostatic intraepithelial neoplasia. The obstructive and irritative symptoms were suggestive of BPH. History of bone pain, backache, haematuria, weight loss and dysuria were also taken which are suggestive of carcinoma prostate.

Blood samples for PSA estimation were taken at the time of presentation before performing any prostatic manipulations and surgical procedure including catheterization.

Physical examination was done which included general, systemic and digital rectal examination (DRE). DRE was done to note the size, shape, and capsule involvement, presence of nodules, induration, and immobility of rectal mucosa, consistency and obliteration of median or lateral sulcus of prostate. A clinical diagnosis of BHP or carcinoma was made and patients were further evaluated to confirm the diagnosis. Suspected cases of BHP underwent for the USG guided Transurethral resection of prostate (TURP) and suspected cases of carcinoma of prostate underwent transrectal prostatic Tru-cut needle biopsy. This was fixed in 10% formalin and sent for histopathological examination to confirm the diagnosis.

Specimens were routinely processed and multiple thin section were taken from each block and stained with Haematoxylin and Eosin stain and Immunohistochemical stain was also performed. A sequential double stain of IHC is used for the simultaneous detection of two different antigens within one tissue section. A primary antibody in applied to the tissue, followed by a horseradish peroxidase (HRP) detection system. A denaturating step in required to eliminate cross reactivity from the application of the second detection system. A second primary antibody in them applied, followed by an alkaline phosphatase (AP) detection system. Follow all staining methods of IHC as per standard protocol. ERG stains nucleus brown in colour and CK5 stains cytoplasm red in colour. For positive control known prostate cancer tissue used (ERG positive & CK negative). For negative controlnormal prostate tissue (CK5 positive) with blood vessels (ERG positive endothelial cells of blood vessels) and also ERG & CK positive PIN with prostate cancer. The statistical analysis was done using

SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software.

Results

Out of 30 patients, Histologically 11 were having benign hyperplasia prostate (36.7%), 10 were carcinoma prostate (33.3%) and rest 9 were prostatic intraepithelial neoplasia (PIN) (30%) (Figure1) BHP cases were maximum in age group 70-79 year (45-.5%) and next most common age group were 60-69 year (36.4%).Similar pattern of distribution was seen in PIN cases Among Carcinoma Prostate maximum number of cases was in age group of 70-79 year (40%). Age group 40-49 year shows least number of cases of carcinoma prostate. In case of benign hyperplasia prostate (BHP), urinary frequency was the predominant symptom in 7 (64%) cases followed by incomplete emptying in 6 (54%), retention of urine in 5 (45%) and nocturia in 5 cases (45%), whereas in carcinoma prostate cases weak urinary stream in 8 cases (80%) was predominant followed by urinary frequency in 5 cases (50%), haematuria and incomplete emptying in 40% each. Retention of urine, urgency, and bone pain are the least mode of presentation in CA prostate each. Among PIN cases, frequency and straining in micturation was predominant symptom in 55% of each cases, followed by weak urine stream in 44%cases, incomplete emptying, retention of urine and intermittency in 22% of each cases (Figure2).

In digital rectal examination (DRE) in BHP cases prostate was firm in 91% of cases. Tenderness in 27% with no case showing nodularity and mucosal involvement on digital rectal examination. Similar findings were felt in PIN cases, except that nodularity was present in 22% cases & hardness of prostate seen in 11.1%. Whereas in CA prostate hardness of prostate with ill defined groove (70%) and nodularity (80%) was predominant DRE finding. Mucosal involvement and mobility restriction in (30.0%) each are also felt on DRE.

Among BHP maximum number of cases 7 (63.6%) lie in PSA range 4-8 ng/ml, 3 cases (27%) lie within normal range of 0-4 ng/ml & only one case (9.0%) have PSA value >12ng/ml.In contrast among carcinoma prostate maximum numbers of cases 8 (80%) have PSA value >12ng/ml, one case show PSA level within normal range. Among PIN cases maximum number of cases 4 (44.4%) lie in 4-8 ng/ml PSA range followed by 3 (33.3%) lying in 8-12 ng PSA range, 1 case (11.1%) has PSA level within normal range & other one (11.1%) has PSA value > 12 ng/ml (Figure3).

On histopathology, 11 were having benign hyperplasia prostate, 10 were carcinoma prostate and rest 9 were prostatic intraepithelial neoplasia (30%). 50% of cases of prostate carcinoma belongs to Gleason's score 5-6 & 50% cases belongs to Gleason's score >6. Immunohisto-chemical stain for ERG and CK 5/6 was performed in all 30 cases. Out of 11 cases of BHP one case shows both intraluminal ERG positivity and CK5/6 positivity. Therefore diagnosed as a case of PIN. Rest of BHP cases are CK5 positive & ERG negative. Therefore 10 cases diagnosed by IHC as BHP cases.

Out of 9 cases of BHP with PIN changes, 2 cases are ERG negative and CK5 positive. Therefore 2cases immunohistochemically diagnosed as BHP cases. 1case show ERG positivity & CK5/6 negativity in tumor areas, therefore immunohisto-chemically diagnosed as case of adenocarcinoma prostate. 6cases show both ERG and CK5 positivity. Therefore 6 cases diagnosed by IHC were PIN cases (Figure4). 10 adenocarcinoma prostate cases show ERG positivity & CK5 negativity in tumor areas. Therefore 10 cases immunohistochemically diagnosed as cases of adenocarcinoma prostate (Table1 & Figure5). Sensitivity and specificity of ERG and CK5 immunostain in detecting ERG positive prostatic adenocarcinoma 100% respectively. Expression of ERG and CK5immunostaining in PIN and Adenocarcinoma prostate varies from 5-6% ERG positive cells to strong 80% positivity (Intensity

+++). No positive correlation is established between PSA level, Gleasons score and ERG and CK5 coexpression in PIN and Adenocarcinoma prostate cases. Maximum number (33.3%) of cases of PIN and Prostate Cancer patients showing high ERG expression have PSA value >12ng/ml. Strong ERG positivity is found in prostatic cancers with gleasons score 5/6.

In our study on follow up period of 6months one ERG positive prostate cancer patient has expired and another one is on chemotherapy. Rest of patients are alive and and on medicinal treatment.

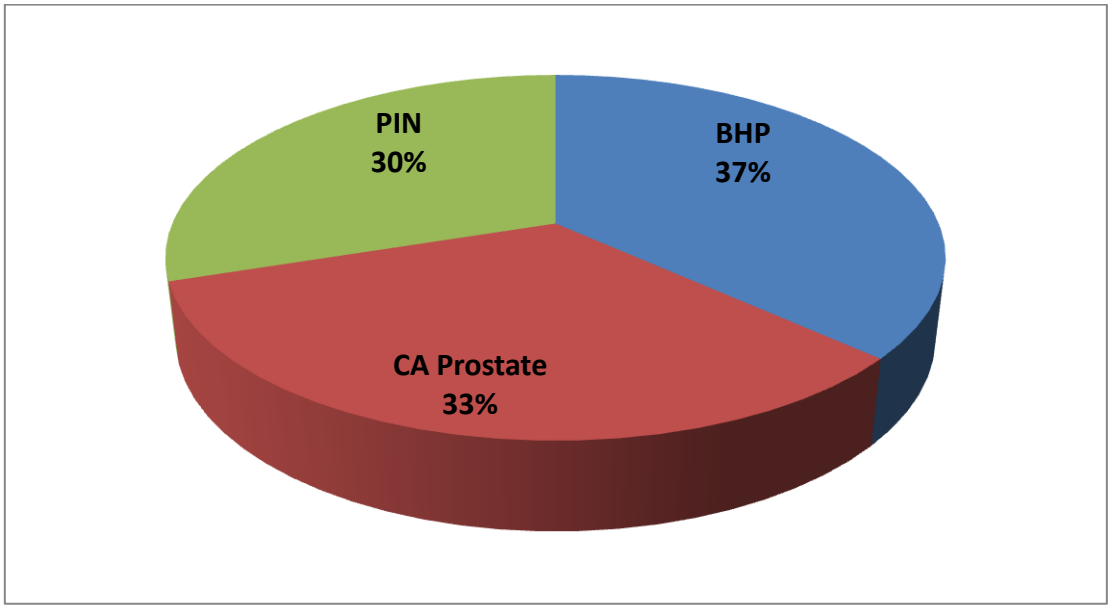

Figure1: Distribution of cases BHP, PIN and carcinoma prostate

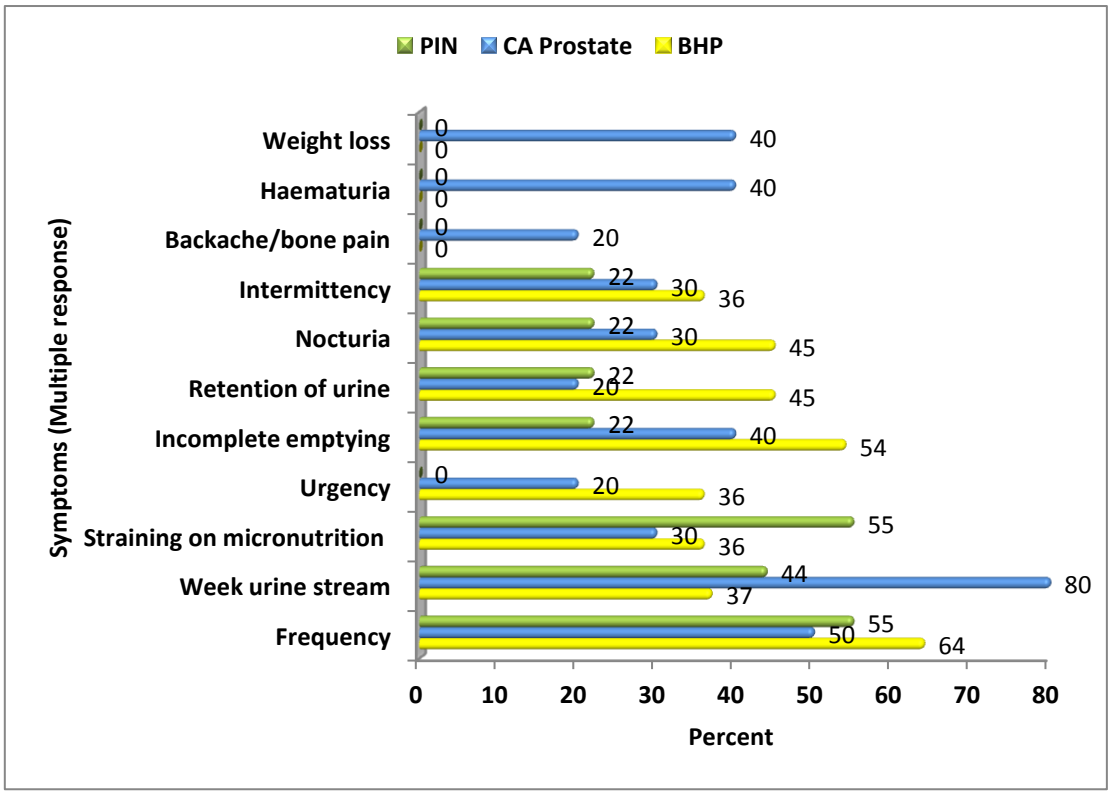

Figure 2: Symptom of BHP, CA prostate and PIN cases

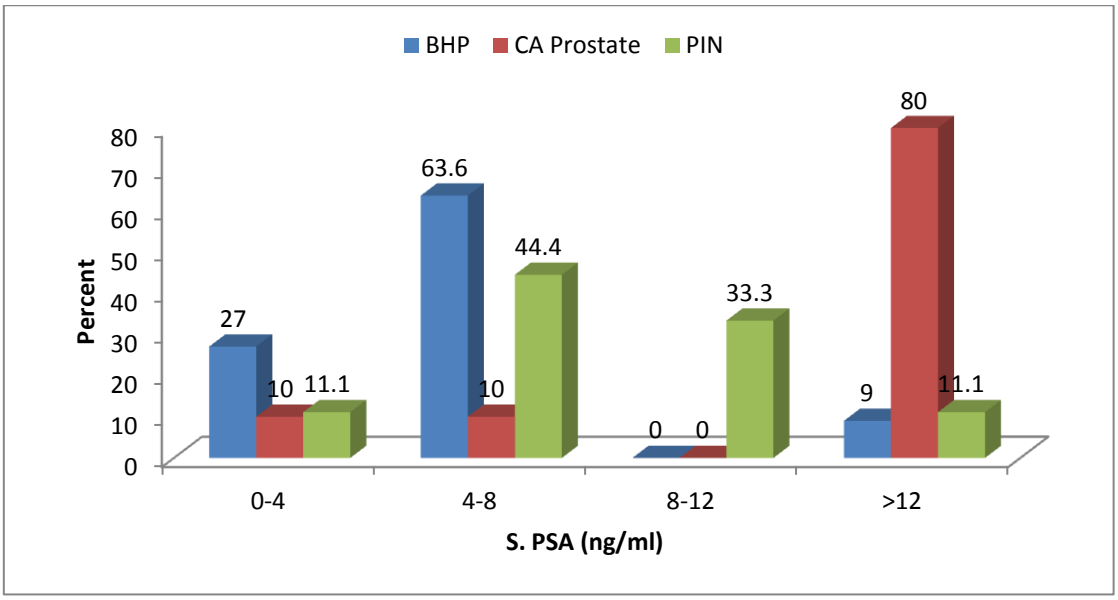

Figure 3: Serum PSA profile in cases of BHP, CA prostate and PIN.

Table 1: Comparison of Histopathological Diagnosis and Final Diagnosis after ERG and p63 immunohistochemical evaluation

Histopathological diagnosis	Final Diagnosis					
	BPH (n=12)		PIN (n=7)		Ca Prostate (n=11)	
	No.	%	No.	%	No.	%
BPH (n=11)	10	90.90	1	9.10	0	0.00
PIN (n=09)	2	22.22	6	66.67	1	11.11
Ca Prostate (n=10)	0	0.00	0	0.00	10	100.00

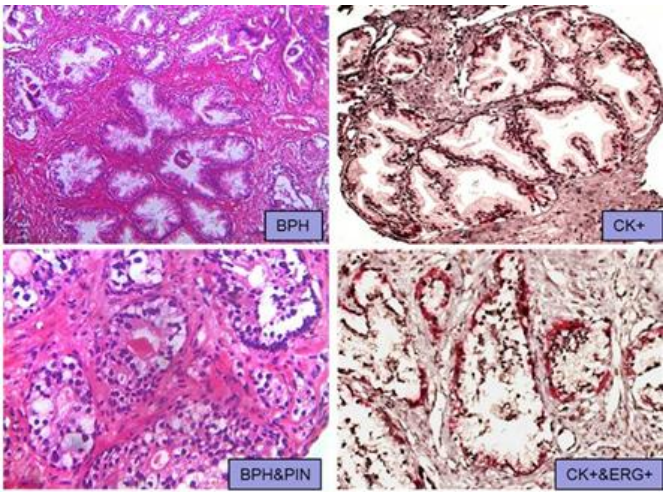

Figure 4- BPH section shows hyperplastic glands (H&E stain,X10) and positive for CK5 (IHCstain,X10) , BPH with PIN showing basal cell layer lined by atypical cells with Stratification, crowding and prominent nucleoli (H&E stain,X20) and positive for CK &ERG (IHC stain,X10).

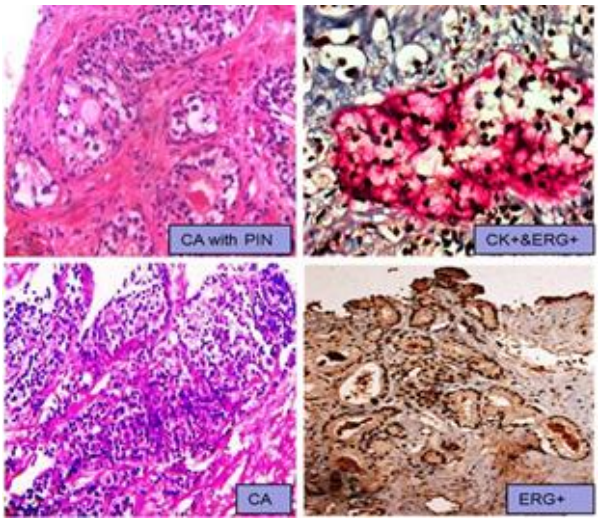

Figure 5- Carcinoma with PIN, few glands showing atypica with Stratification, crowding and prominent nucleoli along with malignant glands (H&E stain,X20) and positive for CK &ERG (IHC stain,X10),Carcinoma showing malignant gland arranged in sheets as well as in glandular pattern 9H&E stain,X20) and positive for ERG (IHC stain,X10).

Discussion

In present study BHP cases were observed maximum in the age group 70-79 years 45.5%, followed by age group of 60-69 years (36.4%). In carcinoma prostate the age group of 70-79 years constituted maximum number of cases 40%. PIN in our study was seen maximum in the age group 70-79 years (44.4%).Patient's symptoms profile in our study shows that in BHP cases urinary frequency, incomplete emptying, retention of urine, nocturia, straining on micturation, in order of frequency were the presenting symptoms. Similar clinical manifestation was mentioned by Riehmann and Bruskewitz [4] in BHP cases.

In our study, obliteration of median groove and nodularity with or without hardness of Digital rectal examination emerged as the most effective predictor of prostate cancer, mobility restriction and rectal mucosal involvement were present in 265.6% and 33.3% of the total carcinoma prostate cases, showing evidence of locally advanced cancer, was consistent with finding of Walsh and Jewett et al[5]. In PIN cases, DRE findings was in the pattern of BHP, only one case on, palpation shows modularity on rectal examination. Our finding is consistent with rectal studies done by Keetch DW et al[6], Davidson D et al [7], that there is no statistically significant difference between BHP and PIN with respect to DRE.

Kovi et al [8] and Transco [9] have shown that prostate intraepithelial neoplasm (PIN) is more often found in cancerous prostate gland than in benign gland. The pre-malignant nature of PIN and particularly its spatial relationship to invasive carcinoma prompted the study of the role of repeated US guided prostate biopsy in men who had PIN identified on a previous needle biopsy of a palpable prostatic abnormality. PIN is a true premalignant lesion or only a tumour associated condition as it is difficult to determine as it is almost impossible, with all available imaging technique, to demonstrate histologically the progression of PIN to invasive cancer. Another point of controversy is the management of these patients, particularly those with high grade PIN. Although a strong association with invasive cancer has been reported in many studies, it is generally agreed that therapy should be deferred until carcinoma is clearly demonstrated, in the study made by Abosief S et al [10] there was no cancer in 14% of patients with high grade PIN after 2 year of follow up. It is generally believed that, although slow growing cancer cannot be excluded, the benign course of these cases suggest the need to clear diagnosis before proceeding with treatment and its potential complications. In our study on symptomatic profile of PIN was similar to BHP cases, but its diagnosis is made histologically so its diagnosis is important as possibility of cure is highest at stage of PIN associated with grades of carcinoma. Its identification on biopsy specimen requires close surveillance.

Weintein & Epstein [11] reported that the serum PSA was elevated in 90% of patients with High Grade PIN and cancer compared to only 50% of them High Grade PIN without cancer. They concluded that serum PSA measurement may be useful in distinguishing which patient with PIN has cancer. In this study no definitive correlation between PIN and carcinoma patient.

Gaudin PB et al [12] concluded in his study, that High Grade PIN on TURP is relatively uncommon and is diagnosed in the elderly population. Patients with PIN on TURP appears to be at increased risk of developing prostatic carcinoma, although not to the same degree as patient with PIN on needle biopsy. Bankhoff, Remberer K & Associates [13] concluded that PIN is considered most likely precursor of clinically significant prostate cancer. Biopsy remain the only definitive method of detecting these pre-malignant lesion. Its identification in biopsy specimen warrants close surveillance with repeat biopsy. Similar findings are also observed in this study.

Early detection of prostatic cancer, using clinically sensitive procedure or tumour marker (PSA) is of prime importance. However the choice of therapeutic intervention for prostate cancer at the time of diagnosis is largely dependent on

clinical and pathological staging and prediction of the degree of aggressiveness of the disease.[14] In this study maximum numbers of carcinoma prostate cases (90%) have PSA value >12ng/ml, only 10% cases show PSA level within normal range.

Furusato et al [3]have shown that ERG oncoprotein monoclonal antibody can detect the presence of ERG oncoprotein with a very high degree of specificity in about 65% of all patents with prostatic cancer. In addition, there appears to be no sign of ERG oncoprotein in the (benign) epithelial cells of men who do not have prostate cancer.

Today, a multiplex IHC cocktail has been developed which combines ERG with a basal cell marker (CK5) to help with there critical diagnoses, Shah explains[15] clinical usefulness of the multiplex IHC test, "CK5 and ERG in combination give the pathologist the ability to determine how aggressively the patient should be followed up, or even if a re-biopsy may be required. CK5 helps to define the integrity of the basal cell layer, so the pathologist can confirm the existence of high grade prostatic intraepithelial neoplasia (HGPIN); even if cancer is not found in the biopsy, ERG positive HGPIN gives strong support for the existence of prostatic adenocarcinoma within a few millimeters of the biopsy due to a "field affect". Regarding diagnostic of ASAP, "The CK5 and ERG multiplex IHC cocktail also increase confidence in diagnoses of ASAP, since ERG positive samples are almost certain to be cancer".

In this study, Out of 11 cases of BHP one case show both intraluminal ERG positivity and CK5/6 positivity. Therefore diagnosed immunohistoc-hemically as a case of PIN. Rest of BHP cases are CK5 positive & ERG negative. Therefore diagnosed by IHC as BHP cases.Out of 9 cases of BHP with focal PIN changes. 2 cases are ERG negative and CK5 positive. Therefore immunohistochemically diagnosed as BHP cases. 1cases show ERG positivity & CK5/6 negativity in tumor areas, therefore immunohistochemically diagnosed as cases of adenocarcinoma prostate. 6cases show both ERG and CK5/6 positivity. Therefore diagnosed by IHC are PIN cases.10 cases of adenocarcinoma prostate show ERG positivity & CK5/6 negativity in tumor areas. Sensitivity and specificity of ERG & CK5/6 immunostain in detecting ERG positive prostatic adenocarcinoma being 100% respectively.

Expression of ERG and CK5/6 immunostaining in PIN and Adenocarcinoma prostate varies from 5-6% ERG positive cells to strong 80% positivity.No positive correlation is established between PSA level, Gleasons score and ERG & CK5 coexpression in PIN and Adenocarcinoma prostate cases in this study.

Maximum number (33.3%) of cases of PIN and Prostate Carcinoma patients showing high ERG expression have PSA value >12ng/ml.

Strong ERG positivity is found in prostatic cancers (with gleasons score 5/6). ERG positive carcinoma prostate cases may be associated with increased risk of tumor progression, it may help to decide treatment options ranging from medical treatment to aggressive management with radiation, chemotherapy or surgery.

In our study on follow up period of 6months one ERG positive prostate cancer patient has expired and another one is on chemotherapy. Rest of patients are alive and and on medicinal treatment.

Conclusion

Thus staining for ERG and CK5 has great utility in resolving diagnostic problems of PIN and adenocarcinoma prostate cases that arise in prostatic needle biopsies containing small foci of suspicious cells. Accurate morphological assessment of prostatic biopsies remains the enduring gold standard of histopathological diagnosis. However immunohistostaining for (ERG and CK5) is an important adjunct to the morphological diagnosis and predictive analysis of prostate biopsy specimens.

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